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Patent
Attorney's Docket No. ~~002000-293~~

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application Branch

In re Patent Application of	)	
	)	
Per HOFVANDER et al	)	Group Art Unit: ~~1804~~ 1803
	)	
Application No.: 08/070,455	)	Examiner: D. Fox
	)	
Filed: June 9, 1993	)	
	)	
For: GENETICALLY ENGINEERED MODIFICATION OF POTATO TO FORM AMYLOPECTIN-TYPE STARCH	)	application Branch

DECLARATION PURSUANT TO 37 C.F.R. §1.132

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

I, Per T Persson, hereby declare and state the following:

1. I am one of the inventors of the above-cited application.

2. The following are experiments conducted under my direction to characterize the genetically modified starch of the amylopectin type:

Serial No. 08/070,455

3. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United Stated Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

25-10-1994
Date

Per T Persson

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Characterisation of genetically modified starch of amylopectin type

Non-mutant or normal starch granules are composed of amylose and amylopectin. The amylose is mainly linear and the amylopectin is heavily branched. The main properties of amylose and amylopectin are shown in **Table 1**. Differences in properties between the normal potato starch and the genetically modified potato starch (GMS) of amylopectin type will be described below following **Table 1.**

Property	*Amylose*	*Amylopectin*
General structure	Essentially linear	Branched
Color with iodine	Dark blue	Purple
λ_{max} of iodine complex	~650 nm	~540 nm
Iodine affinity	19–20%	<1%
Average chain length (glucose residues)	100–10,000	20–30
Degree of polymerization (glucose residues)	100–10,000	10,000–100,000
Solubility in water	Variable	Soluble
Stability in aqueous solution	Retrogrades	Stable
Conversion to maltose by crystalline β-amylase	~70%	~55%

Table 1. Properties of amylose and amylopectin components of starch[1] .

1. Colour with iodine: (see Table 1)

From **Figure 1** it can be clearly seen that the normal potato starch colours in blue and the GMS colours in brown by iodine.

Normal potato starch *GMS of amylopectin type*

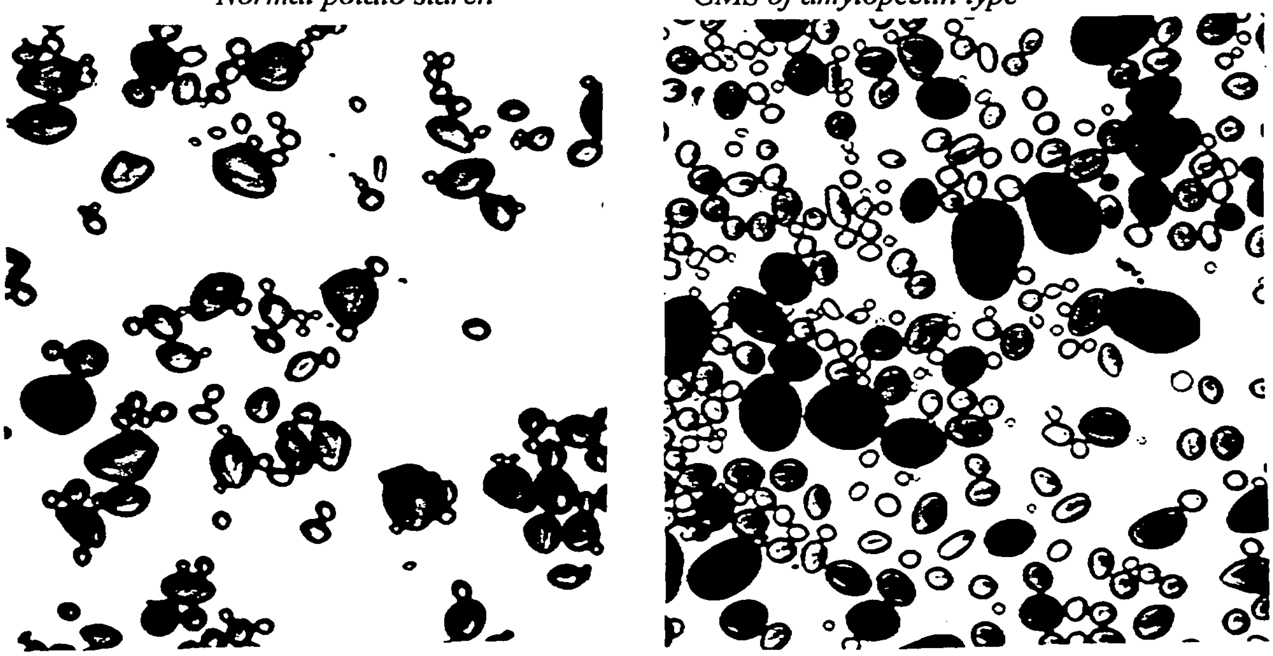

Figure 1. Micrographs of native starch granules stained by iodine

2. Iodine affinity (see Table 1)

The iodine binding capacity and the colour with iodine are used as a criteria and even as a definition for amylose. Iodine staining is a measure of the average chain length. Using the iodine binding capacity to determine the amylose content leads to overestimation if the starch contains branched material with long side chains and to underestimation if the short linear chains are present.[2,3] Potato starch amylopectin has longer side chains than cereal starches such as corn and wheat[4].

The amylose content was determined by iodine binding and the normal potato starch had a amylose content ranging from **20-25%** whereas the GMS ranged from **6-9%**. The GMS genotype presented in **Figures 1, 4 and 5** had an estimated amylose content of **7,5%.**

3. General structure - Average chain length (see Table 1)

The degree of branching was determined using high field ^{1}H-NMR spectroscopy[5]. The chain length between branching points (CL) of potato starch (average of both amylose and amylopectin) was determined to **31,6** and the average of the GMS was **25,6** for the genotype presented in **Figures 1,4 and 5.** The decrease in CL signifies an increase in branching.

4. Stability in aqueous solution

Starch can be dissolved by cooking. The amylose and amylopectin varies in their stability on storage at room temperature after cooking as seen in **Table 1**. The stability of 4% starch pastes were detected by changes in the clarity and paste thickness on long term storage at +5°C. Results are shown in **Figures 2 and 3.** The GMS remains more clear and stable than normal potato starches during storage.

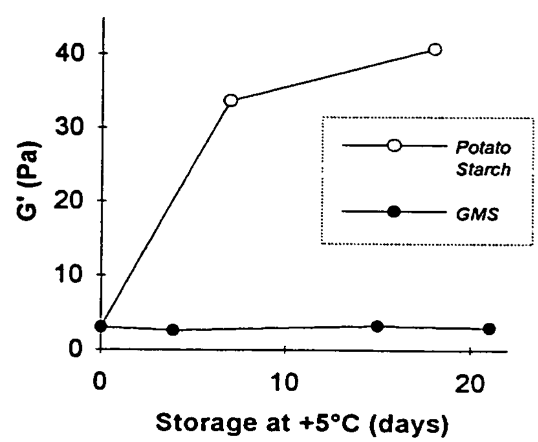

Figure 2. Changes in G' on storage at +5° C. The elastic modulus (G') is an indication of paste firmness. Increased G' is associated with retrogradation.

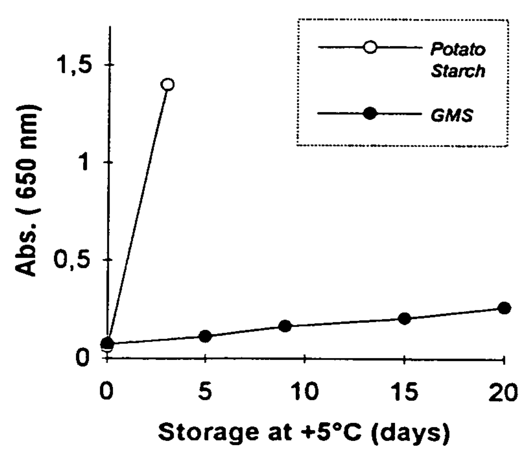

Figure 3. Changes in paste clarity on storage at +5°C. The opacity is measured by the light absorbance at 650nm.

5. Swelling patterns

When the native starch granules are cooked they swell and form gellike particles. **Figure 4** shows a typical micrograph of a section of a 8% potato starch pastes cooked to 70° C[6]. The walls of the swollen granules are seen in violet and the dark blue areas are amylose which has leached out from the gel phase. The GMS shows a completely different swelling pattern on cooking. **Figure 5** depicts a GMS paste at 8% cooked to 65°C. At this temperature some granules are still in the process of swelling and those can be seen as dark brown particles. One important structural difference is that the granules of the GMS are directly disintegrated and dissolved after swelling whereas the normal potato starch remains in the form of gel particles. Another difference is the staining intensity, the iodine affinity of amylose (see **Table 1**) is greater than the affinity of amylopectin leading to a weaker staining of the GMS.

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[1]Shannon J.C. and Garwood D.L. (1984) in Starch: Chemistry and Technology. eds. Whistler R.L., Bemiller J.N. and Paschall E.F. Academic Press, Orlando, San Diego. New York. London. Toronto. Montreal. Sydney. Tokyo. 26-86
[2]Banks W. and Greenwood C. T. (1975) Starch and its Components. University Press. Edinburgh
[3]Takeda Y., Hizukuri S. and Juliano B.O. (1987) Carbohydrate Research, 168, 79-88
[4]Kalichevsky M.T., Orford P.D. and Ring S.G. (1990) Carbohydrate Research, 198, 49-55
[5]Gidley M. J. (1985) Carbohydrate Research, 139, 85-93
[6]Svegmark K. and Hermansson A.-M. (1991) Food Structure, 10, 117-129

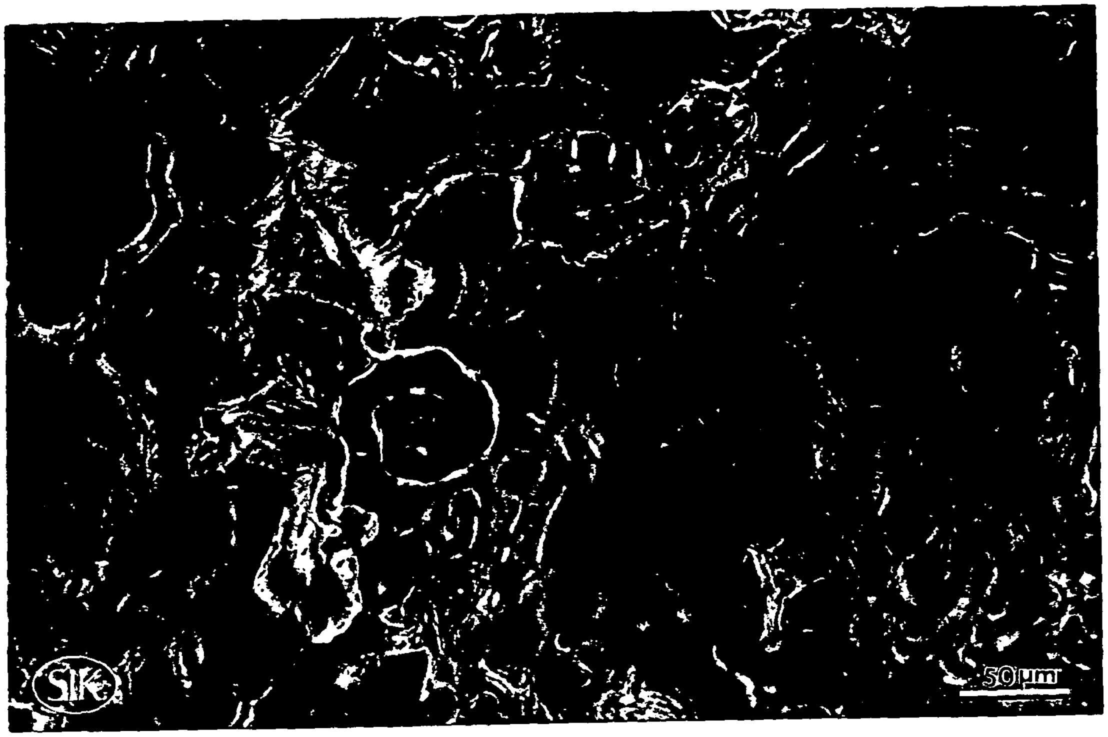

Figure 4. Cryosection of a 8% potato starch paste heated to 70°C.

Figure 5. A cryosection of a 8% genetically modified potato starch paste heated to 65°C.

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